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A DNA Probe Detecting Multiple Haplotypes of the Human Y Chromosome

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SUMMARY

We have characterized a DNA probe (49f) that detects about 15 Y-specific *Taq*I bands corresponding to a low-copy number sequence. Five of these bands, each representing a single DNA fragment, can either be present, absent, or variable in length. Familial segregation studies have shown that the variations of these five fragments are inherited in a Mendelian fashion and strictly Y-linked. A survey of 44 male individuals indicated that the five variable *Taq*I fragments detected by probe 49f can be considered as five independent allelic series. Each series represents the different and mutually exclusive allelic forms observed for a single DNA fragment. A total of 16 haplotypes, each defined by a different combination of the various forms of each of these five restriction fragment length polymorphisms, were observed among the 44 scored individuals. These *Taq*I restriction polymorphisms are not observed with other restriction digests and have therefore been attributed to point mutations. The five polymorphic fragments map to Yq11, a region that does not recombine with the X chromosome and are therefore not redistributed. This implies that an apparently independent reassortment of one of these series with respect to the others can be explained only on the basis of mutations that occurred several times (or reverted) during evolution of the Y chromosome. However, an examination of the different combinations of two or more allelic series suggests that some alleles are not randomly distributed and raises the possibility of establishing a genealogy of the human Y chromosome.

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INTRODUCTION

The use of restriction fragment length polymorphisms (RFLPs) has been proposed for the construction of a human genetic linkage map [1]. This approach has already provided major results in diagnosis of some human heritable diseases [2–6] and in establishing linkage maps of human chromosomes [7, 8]. RFLPs have also become a powerful tool in human and animal population genetics. In that respect, molecules like the mitochondrial DNA (mtDNA) or the mammalian Y chromosome, which carry markers that are not redistributed at meiosis, are of particular interest.

The several thousands of copies of mtDNA present in a mammalian cell appear to be maternally inherited and, paradoxically, homogeneous within an individual organism in spite of their supposedly fast rate of evolution (reviewed in [9, 10]). Because of its small size, animal mtDNA can be observed as a whole at the molecular level. Several groups took advantage of this favorable situation as well as the ease of preparation of mtDNA [11] and their results have shed some new light on population genetics [10, 12–14] and evolution of closely related species [9, 15].

On the other hand, the Y chromosome represents the specific paternal contribution to the male genome and use of DNA polymorphisms from the mammalian Y chromosome would provide a powerful and complementary alternative to the mtDNA studies. At least a part of the Y chromosome is normally transmitted as a single haploid entity and a neutral mutation arising in this chromosomal part will be transmitted to all the male progeny. Neutral mutations may accumulate fast on this chromosome, which appears to be the least conserved among the mammalian chromosomes [16]. Furthermore, because of size differences of three orders of magnitude, the Y chromosome represents a potentially much larger source of variants than mtDNA. Until recently, progress has been hampered by the lack of valuable tools for studies of Y DNA polymorphisms. Such tools are becoming available with the isolation of numerous human Y-specific DNA probes [17–19], and Y DNA polymorphism studies have already been initiated in the mouse species [20, 21].

As compared to mtDNA, the major drawback of the Y chromosome in population studies is that it can only be partially analyzed at particular sites (a few kilobases [kb]) with probes detecting only a very limited number of variants. To delineate an overall picture of the Y chromosome of an individual, numerous probes will be needed. We have characterized a probe detecting a low-copy number sequence in about 15 Y-specific *Taq*I fragments spanning a region of about 100 kb. Several of these *Taq*I fragments are polymorphic, and the analysis of the various Y-specific RFLPs is given in this report.

MATERIALS AND METHODS

Origin and Preparations of Human DNAs

DNAs came from a sampling of unrelated Parisian individuals [22] and from voluntary donors selected preferentially for their various ethnic origins; about 50 are Caucasians, and an additional sample of 15 blacks and 10 Mongoloids were also studied. High

molecular weight DNA was extracted from livers, fetal placentas, or peripheral blood lymphocytes, according to published procedures [23, 24].

Y-specific DNA Probes

A set of probes originating from cosmid 49 of our collection [17, 18] and belonging to locus DYS1 [25] has been isolated by subcloning various DNA fragments of the cosmid insert. Probes p49z and p49f are *Eco*RI fragments of 1.6 and 2.8 kb, respectively, whereas p49a is a 0.9-kb *Xba*I-*Bam*HI fragment. The *Bam*HI site represents an extremity of the cosmid insert and may have been artificially created during the cloning procedure in cosmid pJB8 [18]. Positions of the probes relative to each other are indicated in panel A of figure 1.

Restriction Digestions, Electrophoresis, and Transfer of DNA

Restriction endonuclease digestions of human genomic DNA were carried out as specified by the manufacturers (Amersham, Boerhinger-Mannheim). Complete digestion of DNA samples was monitored on aliquots by mini-gel horizontal electrophoresis after 5 hrs of incubation or more. Ten micrograms of restriction-digested human DNA were separated by electrophoresis on 0.8% agarose horizontal gels in 2.5 mM EDTA, 90 mM Tris-borate, pH 8.3. DNA was partially depurinated by soaking the gel for 20 min in 0.25 N HCl, then denatured for 30 min in 0.5 N NaOH, 1 M NaCl, and neutralized for 30 min in 0.5 M Tris, pH 7.5, 1 M NaCl. Denatured DNA was transferred from the gel to

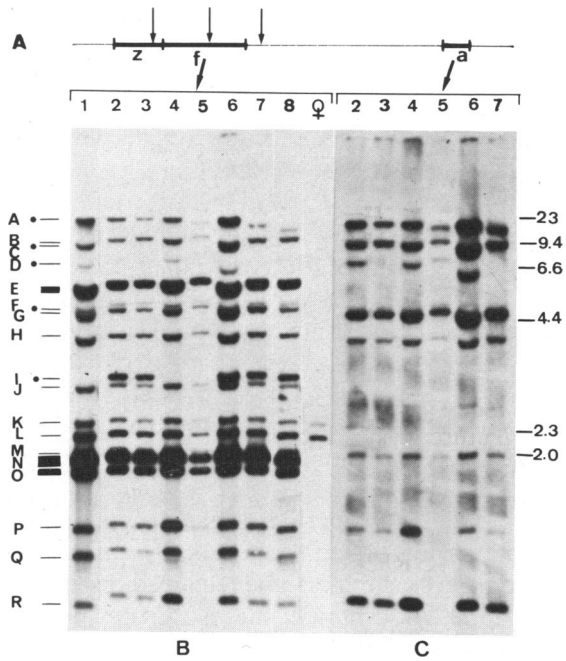

FIG. 1.—*Panel A:* Mapping of probes from cosmid 49. *The three upper arrows* localize the *Taq*I sites from fragments N and O. This localization has been obtained from restriction mapping studies on cosmid 49 (unpublished results). *Panel B:* Hybridization of probe 49f to *Taq*I digests of DNA from eight unrelated males (1–8) and one female. *Panel C:* Hybridization of probe 49a to the same *Taq*I digests of males 2–7 as in panel B.

Zetabind membrane (Microfiltration Products Division) in 20 × SSC according to Southern [26]. Filters were dried at room temperature and baked at 80°C for 1 hr.

Hybridization

Baked filters were washed for 1 hr in 0.1 × SSC, 0.5% SDS at 65°C for background reduction and prehybridized overnight at 42°C in 50% formamide, 5 × SSC, 0.2% Ficoll 400, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 mM sodium phosphate, pH 6.7, and 500 μg/ml denatured sonicated salmon sperm DNA. Prehybridized filters were hybridized overnight at 42°C in 50% formamide, 5 × SSC, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 20 mM sodium phosphate, pH 6.7, 10% dextran sulphate, and 100 μg/ml denatured sonicated salmon sperm DNA with 5–7 × 10^7 cpm of denatured ^{32}P-labeled DNA probe per blot. Probes were nick-translated according to Rigby et al.[27] to a specific activity of approximately 3–4 × 10^8 cpm per μg of DNA. Hybridized filters were washed twice for 30 min each at 42°C in 2 × SSC, 0.1% SDS, dried, and exposed for autoradiography at −70°C with Kodak XAR-5 films backed by intensifying screens.

RESULTS

Detection of Y-specific DNA Polymorphisms

Panel B of figure 1 shows a typical hybridization pattern of probe 49f on *Taq*I digests of genomic DNA from different male individuals along with one female DNA. Only bands that could be observed reproducibly in several identical digests of the same sample and after several hybridization experiments were considered in this study. Under nonstringent washing conditions (see MATERIALS AND METHODS), this probe detects up to 18 *Taq*I bands, schematized on the left of figure 1. Among those 18 bands, band K and L were also present in female digests and have been assigned to an autosome (unpublished result), whereas all the remaining bands were Y-specific. These Y-specific fragments have been mapped to a subregion of Yq11 [28]. Restriction mapping data from cosmid 49 (fig. 1) indicates that the cognate sequences (strictly identical to probe 49f) are present in bands N and O, while the fragments present in bands A–J and in bands M, P, Q, and R correspond to cross-hybridizing material. Thus, probe 49f detects a family of moderately repeated sequences present in restriction fragments of different sizes. Bands A and D exhibit variation in size or are absent, and bands C, F, and I are either present or absent from the digests. Only a single form of each of the five variable bands is present in a *Taq*I digest of human male DNA, indicating that each band corresponds to a unique fragment.

To eliminate the possibility of partial digestions, the same DNA samples were digested with increasing concentrations of *Taq*I restriction enzyme. The resulting hybridization patterns remained unchanged, especially with regard to the variable fragments (data not shown), suggesting that this variability was due to restriction fragment length polymorphisms. This hypothesis was confirmed by familial segregation studies. All the males of a same family displayed an identical hybridization pattern, with one single form of each of the five variable fragments per family. This result was obtained for all the families studied and three of them are shown in figure 2.

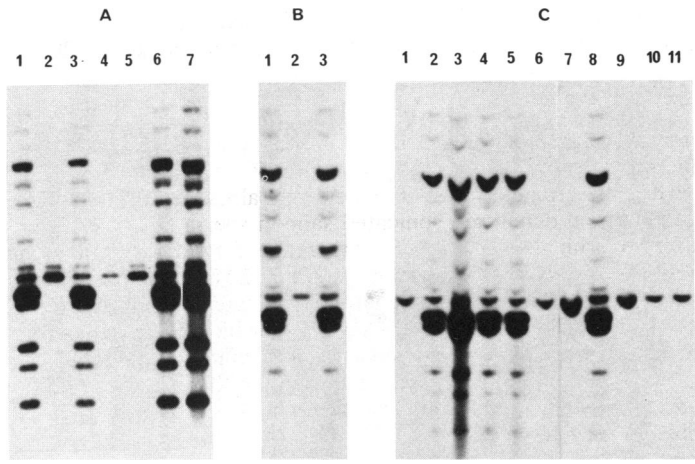

FIG. 2.—Hybridization of probe 49f to *Taq*I digests of DNA from individuals of three families. Males of family A display haplotype *A3-C1-D1-F1-I1* (see fig. 3 and table 1); males of family B display haplotype *A3-C1-D2-F1-I1*; males of family C display haplotype *A3-C0-D0-F1-I0*.

Several RFLPs Define Multiple Haplotypes of the Human Y Chromosome

A detailed analysis of the RFLPs suggested by familial studies was performed by a survey of 44 unrelated males. The different allelic forms of the five polymorphic fragments are illustrated in figure 3 and the results of the survey are scored in table 1.

A preliminary study had shown that the common haplotypes are present in all the three racial groups sampled and that no particular combination is specific of a particular race; so, the 44 unrelated males retained for this study are only those for whom all the variable bands have been unambiguously observed.

Fragment A has been observed in four different sizes (*A1*, *A2*, *A3*, and *A4*) and was also undetected in two instances (*A0*). The presence of *A1*, *A2*, *A3*, and *A4* was mutually exclusive and consistent with different allelic forms of the same restriction fragment. Most of the forms (i.e., *A0*, *A1*, *A2*, and *A3*) have been observed in more than one pattern and are apparently independent of the other four RFLPs and, together with *A4*, define an allelic series.

Fragment C is either present (*C1*) or undetected (*C0*) in the digests. *C1* appears highly preferentially associated with *A3* (in one instance with *A2*) and exclusively with *I1*. However, *I1* can also be found with *CO*, indicating that RFLPs of fragments C and I are independent.

Fragment D has been observed in two different and mutally exclusive sizes (*D1* and *D2*) or was not detected (*D0*). *D2* appears exclusively in a single combination, but is associated with allelic forms (*A3*, *C1*, *F1*, *I1*) which occur in other associations. *D1* and *D0* are scattered in various combinations and appear to define an allelic series with *D2*. When fragment D is detected (*D1* and *D2*), hybridizing intensities of bands A and B are comparable, whereas *D0* is associated with a relative increase of band B (fig. 1, panel B, lanes 3, 7, and 8). Conversely, bands P and Q are usually of similar intensity in combinations with

Allelic series	A					C		D			F		I	
Alleles	A4	A3	A2	A1	A0	C1	C0	D1	D2	D0	F1	F0	I1	I0
Fragment size (kb)	17	15	12	10	-	8.1	-	7.2	6	-	5.4	-	3.5	-
Number of individuals	2	30	8	2	2	16	28	24	10	10	43	1	28	16

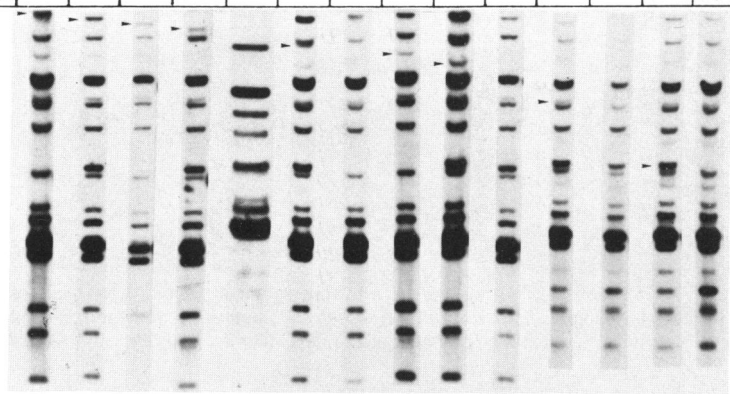

FIG. 3.—The five allelic series detected by probe 49f. *Taq*I hybridization pattern of different individuals representing each one allele. The apparent allelic fragment is *arrowed*. Frequency of each allele among the 44 individuals tested is indicated in footnote to table 1.

the *D1* and *D2* alleles, whereas the *DO* form is associated with a relative decrease of band Q. Moreover, the sum of the sizes of Q and *D1* (respectively, 7.2 and 1.2 kb) roughly equals the size of B (8.5–9 kb). These observations suggest that fragment *D1* and a fragment from band Q could be contiguous and share a *Taq*I site, whose loss may result in *D0* comigrating with fragment B.

Absence of fragment F (*F0*) has been observed only in one instance and must be considered as a rare variant. Fragment I can be either present (*I1*) or absent (*I0*). Although, as mentioned above, *I0* is never associated with *C1*, both forms appear in various combinations and define an allelic series.

Thus, analysis of restriction patterns of 44 males allows us to propose that probe 49f detects five apparently independent allelic series. Each series consists of the different and mutually exclusive allelic forms observed for a single DNA fragment. Among the 44 individuals analyzed, 16 different combinations of the five RFLPs have been found and are indicated in table 1, with the number of cases scored for each combination. Since all the elements of a combination are syntenic and separated on the chromosome by several kilobases at least (see below), it can be considered that each combination defines a haplotype of the human Y chromosome.

Analysis of the Y-specific RFLPs

A molecular interpretation of the multiple RFLPs detected by probe 49f cannot be directly deduced from the complex hybridization pattern of this probe. We therefore hybridized probe 49a, 6 kb distant from 49f (fig. 1), to the same *Taq*I digests. A subset of the hybridization bands detected by p49f hy-

TABLE 1

HAPLOTYPES OF THE HUMAN Y CHROMOSOME OBSERVED AMONG 44 UNRELATED INDIVIDUALS

HAPLOTYPES	ALLELES					NO. INDIVIDUALS OBSERVED	OBSERVED FREQUENCY (%)	RANDOM SEGREGATION FREQUENCY (%)*
	A	*C*	*D*	*F*	*I*			
I	*A0*	*C0*	*D0*	*F1*	*I1*	1	2.3	0.4
II	*A0*	*C0*	*D1*	*F1*	*I1*	1	2.3	1.0
III	*A1*	*C0*	*D0*	*F1*	*I0*	1	2.3	0.2
IV	*A1*	*C0*	*D0*	*F1*	*I1*	1	2.3	0.4
V	*A2*	*C0*	*D0*	*F1*	*I1*	2	4.5	1.6
VI	*A2*	*C0*	*D1*	*F0*	*I1*	1	2.3	0.1
VII	*A2*	*C0*	*D1*	*F1*	*I0*	3	6.8	2.2
VIII	*A2*	*C0*	*D1*	*F1*	*I1*	1	2.3	3.9
IX	*A2*	*C1*	*D0*	*F1*	*I1*	1	2.3	0.9
X	*A3*	*C0*	*D0*	*F1*	*I0*	2	4.5	3.5
XI	*A3*	*C0*	*D0*	*F1*	*I1*	2	4.5	6.1
XII	*A3*	*C0*	*D1*	*F1*	*I0*	8	18.2	8.4
XIII	*A3*	*C0*	*D1*	*F1*	*I1*	3	6.8	14.7
XIV	*A3*	*C1*	*D1*	*F1*	*I1*	5	11.4	8.4
XV	*A3*	*C1*	*D2*	*F1*	*I1*	10	22.7	3.5
XVI	*A4*	*C0*	*D1*	*F1*	*I0*	2	4.5	0.6

NOTE: Each haplotype results from the combination of a single allelic form of each of the five independent RFLPs shown in figure 2.

* This frequency has been derived from the observed allelic frequencies assuming independent reassortment of each series. Allelic frequencies are obtained from the values in figure 3 and are as follows: *A0*, .454; *A1*, .0454; *A2*, .1818; *A3*, .6818; *A4*, .0454. *C0*, .6364; *C1*, .3636. *D0*, .2273; *D1*, .5454; *D2*, .2273. *F0*, .0227; *F1*, .9773. *I0*, .3636; *I1*, .6364.

bridizes also to probe 49a (fig. 1, panel C). Autosomal bands K and L, bands N and O (cognate to 49f), as well as the polymorphic fragment I are no longer detected. Interestingly, the larger polymorphic fragments (A, C, and D) show exactly the same allelic variations as with probe 49f. Since probes 49f and 49a do not crossreact, these large fragments each contain at least one sequence cross-hybridizing with one of both probes. Band Q does not react with probe 49a, and thus the relative increase of the hybridization signal of band B in *D0* haplotypes can mainly be ascribed to a component reacting only with probe 49f. This may explain why *D0* haplotypes do not exhibit marked differences in hybridization intensities between bands A and B with probe 49a (fig. 1, panel C, lanes 3 and 7).

Sequence 49a is much less repeated than sequence 49f, implying that the original duplications that generated this family did not involve the same element or have been considerably altered. On the other hand, band N remains the only band from the hybridization pattern of figure 1 that is still detected by probe 49z (not shown), indicating that fragment N overlaps the end of the repeated element. Although smaller than the distance between 49f and 49a, fragments H, M, P, and R are detected by both probes. In fragment R, for instance, sequences homologous to one of probes 49f and 49a can be separated by less than 1 kb. This may imply that 49a could represent (or is very close to) the other extremity of the repeated element.

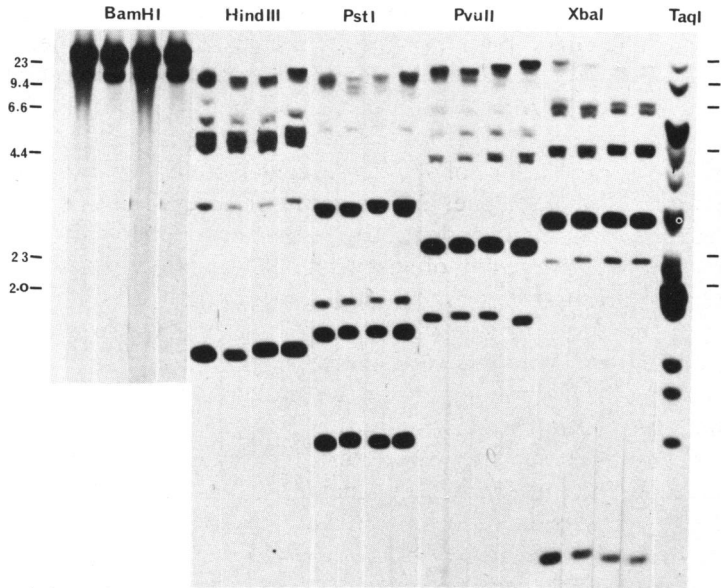

FIG. 4.—Hybridization of probe 49f to several restriction digests of four male individuals with, respectively, haplotypes *XII*, *VII*, *XIV*, and *XV*.

Other restriction digests were also probed with 49f. The results for *Bam*HI, *Hin*dIII, *Pst*I, *Pvu*II, and *Xba*I digests are shown in figure 4. The four samples displayed correspond from left to right to haplotypes *A3-C0-D1-F1,I0*, *A2-C0-D1-F1-I0*, *A3-C1-D1-F1-I1*, and *A3-C1-D2-F1-I1*. No band displacement or supernumerary band could be correlated with any of the *Taq*I polymorphisms, suggesting that these latter are mainly the result of point mutations. An occasional supplementary minor band could be detected in at most one of the digests of the same haplotype and could never be correlated with a similar behavior in other restriction digests from the same sample.

In some instances, an additional copy number polymorphism can be noticed (fig. 1, panel C, band P). Estimations of variations in hybridization intensities need accurate densitometric measurements, which are difficult to perform on complex hybridization patterns and have not been carried out in this first study.

DISCUSSION

We report here the hybridization properties of a probe detecting numerous Y-specific DNA bands. As deduced from restriction analysis of cosmid 49, most of these bands contain sequences cross-hybridizing with probe 49f that appear less intense than the cognate M and O bands on the autoradiograms (figs. 1–3). The occurrence of multiple cross-hybridizing sequences reflects the slightly repetitive character of fragment 49f. In [18], we estimated that a maximum of five fragment copies were present in the cognate band detected by probe 49f in *Eco*RI digests. A similar estimation can be made for bands O and N in *Taq*I digests. Taken together, the fragments detected by probe 49f total 70–

80 kb and thus cover at least a region of the same size that would represent about 0.4% of the euchromatic part of the human Y chromosome.

Some bands (A, C, D, F, and I) are either present or undetectable in *Taq*I digests of genomic DNA from different human males, indicating that each of these bands most probably corresponds to a unique fragment. With the exception of *D0*, the disappearance of one *Taq*I fragment could not be correlated with the appearance of another one, and presence or absence of one of these fragments was essentially independent of the other observable variations. Such a behavior could be the result of insertion/deletion processes or other rearrangements occurring during evolution of the Y chromosome. However, rearrangements, insertions, or deletions are also noticed in restriction digests with different enzymes and we did not observe similar variations of hybridization patterns in the other restriction digests we probed. We therefore propose that appearances (or disappearances) of *Taq*I hybridizing fragments are mainly due to point mutations within the restriction sites, arising in (or leading to) fragments that could comigrate with some major nonpolymorphic bands (like E, N, and O) and remain undetected. This behavior may be illustrated by the putative *D0* fragment comigrating with B. It has been reported that *Taq*I sites are prone to polymorphism [29]. This increased polymorphism frequency has been ascribed to the presence, in their recognition sequence, of the CpG dinucleotide, which is considered as a hotspot for point mutations. The present results illustrate the higher frequency of *Taq*I RFLPs and can account for the proposed mechanism.

Fragments C, F, and I are either present or undetected in the digests, and these two alternate forms define an allelic series for each of fragments C, F, and I. Fragments A and D, which in addition exhibit size variation, can also be undetected. One allele of each of these five RFLPs is present in the genome of any normal male, and their combination permits the definition of a haplotype of the human Y chromosome. The only portion of the Y that is involved in regular recombination processes is restricted to the distal part of Yp [28, 30, 31]. The five RFLPs we describe here are linked within Yq11 [28], a region that does not recombine and are therefore never redistributed. Absence of recombination is further demonstrated by the lack of strictly homologous DNA fragments in the female genome. This implies that an apparently independent segregation of one of these allelic series with respect to the others can be explained only on the basis of mutations that occurred twice or more (or reverted) during the evolution of the Y chromosome. *Taq*I polymorphism can be considered as one aspect of CpG supression in vertebrates [32], in which the high levels of DNA methylation accounts for the deficiency of CpG in general. This implies that in parallel to the evolutionary loss of CpGs there should be a loss of *Taq*I sites and therefore an increase in the size of *Taq*I restriction fragments. Hence, in most cases of *Taq*I RFLPs, the smallest allele should represent the original form. As a corollary, reversions (restoration of *Taq*I sites) must occur rarely and most of the cases of apparent independent segregation mentioned above should be essentially the result of identical mutations that took place at the same site at different times. Since fragments N and O appear to exist in a few copies, the

possibility of several independent mutations in one of those *Taq*I sites is even increased and may have generated fragment I more than once.

All the observed haplotypes could be derived one from another by single mutations. An examination of the different combinations of two or more allelic series (table 1) suggests that some alleles are not randomly distributed but are preferentially associated. The *D2* allele appears in a single haplotype (*XV*) always associated with *C1*, *I1*, and *A3*. Haplotype *XV* is the most frequently observed in our study (23%), although it is only expected at a frequency of 3.5% if in equilibrium. There is generally no or little correlation between the observed haplotype frequencies and the expected frequencies if in equilibrium (table 1). A discrepancy of one order of magnitude or more appears between both expected and observed frequencies for haplotypes *V* and *XVI*, confirming that the haplotypes do not reassort at random.

Such observations outline the possibility of establishing evolutionary relationships among the various haplotypes. However, some additional knowledge is required before a genealogy of the human Y chromosomes can be established: (1) A detailed molecular analysis of the mutations involved in these five polymorphisms would be useful. We suggest that in most cases the smaller allelic forms of the variable *Taq*I fragments will actually represent the original ones. (2) If mutations involved in some of these RFLPs occurred several times, it is probable that several haplotypes are not homogenous. Additional polymorphisms of the Y chromosome will allow a more precise definition of these haplotypes. (3) A population survey on a large scale as well as in particular ethnic groups will bring useful additional information.

This probe represents hitherto the most powerful tool for patriarchal studies in human populations. Because of the numerous mutation events this probe can detect, it may also be of special interest in studying microevolution and polymorphisms in small ethnic groups.

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The Call for Papers will be mailed in time for members to receive it toward the end of May. This document will contain abstract forms and preliminary information about the meeting. Information about how to obtain absentee ballots will also be included as this year's meeting runs over Election Day, November 4. Further information and housing and registration forms will be mailed this summer.